

**What is claimed is:**

1. A method of preparing a polynucleotide having a target sequence from a plurality of oligonucleotides, said method comprising:
  - (a) coupling said oligonucleotides to form a plurality of coupled oligonucleotides, wherein each of said coupled oligonucleotides represents a region of said polynucleotide and shares at least one terminal region of sequence with at least one other coupled oligonucleotide; and
  - (b) assembling said polynucleotide by extension of said coupled oligonucleotides.
2. The method of claim 1 wherein said coupling comprises ligating said oligonucleotides with ligase.
3. The method of claim 2 wherein said ligase is T4 RNA ligase.
4. The method of claim 1 wherein at least one of said oligonucleotides of said coupled oligonucleotides is attached to solid support prior to coupling.
5. The method of claim 1 wherein said coupled oligonucleotides are attached to solid support.
6. The method of claim 1 wherein each of said coupled oligonucleotides is amplified prior to assembling said polynucleotide.
7. The method of claim 1 wherein at least one of said oligonucleotides of said coupled oligonucleotides is blocked at one end prior to said coupling.
8. The method of claim 1 wherein said coupled oligonucleotides comprise pairs of oligonucleotides.
9. The method of claim 1 wherein said extension is carried out using overlap PCR.
10. A library of polynucleotides prepared by the method of claim 1.

11. A method of preparing a polynucleotide having a target sequence from a plurality of oligonucleotides, said method comprising:

- (a) blocking the 3' end of each of said oligonucleotides, except for the oligonucleotide comprising the 5' terminus of said polynucleotide, with a blocking group to form a plurality of blocked oligonucleotides;
- (b) coupling the 5' end of each of said blocked oligonucleotides with the 3' end of a further oligonucleotide of said plurality of oligonucleotides to form a plurality of coupled oligonucleotides, wherein said further oligonucleotide comprises a portion of said polynucleotide immediately 5' to the sequence of said blocked oligonucleotide, wherein each of said coupled oligonucleotides shares at least one oligonucleotide with another coupled oligonucleotide; and
- (c) assembling said polynucleotide by extension of said coupled oligonucleotides.

12. The method of claim 11 wherein said polynucleotide is DNA, RNA, or DNA/RNA hybrid.

13. The method of claim 11 wherein said oligonucleotides comprise from about 10 to about 200 nucleotides.

14. The method of claim 11 wherein said blocking group comprises solid support.

15. The method of claim 14 wherein said solid support is selected from the group consisting of agarose, polyacrylamide, magnetic beads, polystyrene, polyacrylate, controlled-pore glass, hydroxyethylmethacrylate, polyamide, polyethylene, polyethyleneoxy, and polyethyleneoxy/polystyrene copolymer.

16. The method of claim 11 wherein said blocking group is ddUTP-biotin.

17. The method of claim 11 wherein said coupling is carried out using ligase.

18. The method of claim 17 wherein said ligase is T4 RNA ligase.

19. The method of claim 17 wherein said coupling comprises the steps of contacting said blocked oligonucleotide with ligase and cosubstrate to form activated oligonucleotide, washing said activated oligonucleotide to form washed oligonucleotide, and contacting said washed oligonucleotide with said further oligonucleotide and ligase.
20. The method of claim 11 wherein said coupled oligonucleotides are amplified prior to assembling said polynucleotide.
21. The method of claim 11 wherein said extension is carried out using overlap PCR.
22. A library of polynucleotides prepared by the method of claim 11.
23. A method of coupling a first oligonucleotide with a further oligonucleotide, wherein said first oligonucleotide is attached to solid support, comprising contacting said first oligonucleotide with ligase and cosubstrate to form activated oligonucleotide, washing said activated oligonucleotide to form washed oligonucleotide, and contacting said washed oligonucleotide with said further oligonucleotide and ligase.
24. The method of claim 23 wherein said ligase is T4 RNA ligase.
25. The method of claim 23 wherein said cosubstrate is ATP.
26. A method of preparing a library of polynucleotides having a plurality of target sequences from a plurality of oligonucleotides, wherein each of said polynucleotides shares a plurality of predetermined sequence positions occupied by said oligonucleotides, and wherein each of said polynucleotides comprises a different oligonucleotide in at least one predetermined sequence position, said method comprising:
- (a) coupling said oligonucleotides to form a plurality of coupled oligonucleotides, wherein each of said coupled oligonucleotides represents a region of at least one of said polynucleotides and shares at least one terminal region of sequence with at least one other coupled oligonucleotide; and

(b) assembling said library of polynucleotides by extension of said coupled oligonucleotides.

27. The method of claim 26 wherein said coupling comprises ligating said oligonucleotides with ligase.
28. The method of claim 27 wherein said ligase is T4 RNA ligase.
29. The method of claim 26 wherein at least one of said oligonucleotides of said coupled oligonucleotides is attached to solid support prior to coupling.
30. The method of claim 26 wherein said coupled oligonucleotides are attached to solid support.
31. The method of claim 26 wherein each of said coupled oligonucleotides is amplified prior to assembling said polynucleotide.
32. The method of claim 26 wherein at least one of said oligonucleotides of said coupled oligonucleotides is blocked at one end prior to said coupling.
33. The method of claim 26 wherein said coupled oligonucleotides comprise pairs of oligonucleotides.
34. The method of claim 26 wherein said extension is carried out using overlap PCR.
35. The method of claim 26 wherein said plurality of oligonucleotides is derived from a parent set of polynucleotides having at least one common property.
36. The method of claim 35 wherein said common property is sequence homology.
37. The method of claim 35 wherein said common property is enzyme activity.

09013408-03404  
FOR FILING

38. The method of claim 35 wherein said common property is ligand binding.
39. The method of claim 35 wherein said set of polynucleotides is optimized.
40. A method of preparing a library of polynucleotides having a plurality of target sequences from a plurality of oligonucleotides, wherein each of said polynucleotides shares a plurality of predetermined sequence positions occupied by said oligonucleotides, and wherein each of said polynucleotides comprises a different oligonucleotide in at least one predetermined sequence position, said method comprising:
- (a) blocking the 3' end of each of said oligonucleotides, except for the oligonucleotide comprising the 5' terminus of each of said polynucleotides, with a blocking group to form a plurality of blocked oligonucleotides;
  - (b) coupling the 5' end of each of said blocked oligonucleotides with the 3' end of a further oligonucleotide of said plurality of oligonucleotides to form a plurality of coupled oligonucleotides, wherein said further oligonucleotide comprises a portion of at least one of said polynucleotides immediately 5' to said sequence of said blocked oligonucleotide, and wherein each of said coupled oligonucleotides shares at least one oligonucleotide with another coupled oligonucleotide; and
  - (c) assembling said library of polynucleotides by extension of said coupled oligonucleotides.
41. The method of claim 40 wherein said polynucleotide is DNA, RNA, or DNA/RNA hybrid.
42. The method of claim 40 wherein said oligonucleotides comprise from about 10 to about 200 nucleotides.
43. The method of claim 40 wherein said blocking group comprises solid support.
44. The method of claim 43 wherein said solid support is selected from the group consisting of agarose, polyacrylamide, magnetic beads, polystyrene, polyacrylate, controlled-

pore glass, hydroxyethylmethacrylate, polyamide, polyethylene, polyethyleneoxy, and polyethyleneoxy/polystyrene copolymer.

45. The method of claim 40 wherein said blocking group is ddUTP-biotin.
46. The method of claim 40 wherein said coupling is carried out using ligase.
47. The method of claim 46 wherein said ligase is T4 RNA ligase.
48. The method of claim 40 wherein said plurality of oligonucleotides is derived from a parent set of polynucleotides having at least one common property.
49. The method of claim 48 wherein said common property is sequence homology.
50. The method of claim 48 wherein said common property is enzyme activity.
51. The method of claim 48 wherein said common property is ligand binding.
52. The method of claim 48 wherein said set of polynucleotides is optimized.
53. A method of identifying a polynucleotide with a predetermined property, said method comprising, generating a library of polynucleotides according to the method of claim 26, and selecting at least one polynucleotide within said library having said predetermined property.
54. A method of identifying a polynucleotide with a predetermined property, said method comprising, generating a library of polynucleotides according to the method of claim 40, and selecting at least one polynucleotide within said library having said predetermined property.
55. A method of identifying a polynucleotide with a predetermined property, comprising:
  - (a) generating a library of polynucleotides according to the method of claim 26;
  - (b) selecting at least one polynucleotide within said library having said predetermined property; and

